

Study of Preliminary Phytochemical Screening and Antibacterial Activity of *Tribulus terrestris* against Selected Pathogenic Microorganisms

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Abstract

Medicinal herbs have a long history in improving human health and curing various diseases. A wide interest has been made for researchers using herbal material in identification of the active components and verification of their efficiency. We evaluated the aqueous and methanol extracts of *T. terrestris* for the phytochemical content and its antimicrobial activity. Phytochemical screening of the extracts revealed the presence of alkaloids flavonoids, saponins, anthraquinone, terpenoids, tannins, reducing sugar, and Cardiac Glycosides, etc. Disc diffusion method determined antibacterial effect of *T. terrestris* extracts. Methanolic extract have shown better inhibition than aqueous, again all the tested pathogens. Our results indicate that *T. terrestris* has potential therapeutic phytochemicals, which can be used as an alternative medicine for human health.

Keywords: *T. terrestris*; Phytochemical; Antibacterial agent; Broad spectrum

Introduction

Starting from the ancient time, medicinal plants have been used to prevent and treat various health problems. Plants are still an independent source of medication in the contemporary health care delivery system. Their role is twofold in the development of medicines and served as a natural blue print for the development of new drugs [1-3]. In recent years, the growing demand for herbal products has led to a quantum jump in volume of plant materials traded across the countries. Therefore, the use and history of herbs dates back to the time of early man, who had the crudest tools as his implements and use stones to start his fire. They used herbs in their raw and cooked forms to keep fit. Since that time, the use of herbs has been known and accepted by all nations and has been known also as the first art of treatment available to man [4].

Secondary plant metabolite (Phytochemicals), previously with unknown pharmacological activities, has been extensively investigated as a source of medicinal agents [5]. Some of the active principles singly or in combination inhibit greatly the life processes of microbes, especially the disease causing ones. They do this by binding their protein molecules, acting as chelating agents (selective binding polyvalent metal ions so that the latter loses its biological activities), altering their biochemical systems, preventing utilization of available interests to the microorganisms, other causes inflammation analysis of microbial cells [6].

Tribulus terrestris (Puncture Vine, Caltrop, Yellow Vine and Goat head) is a flowering plant of the *Zygophyllaceae* family, native to warm temperature and tropical regions of the old world in Southern Europe, Southern Asia, Africa, India, and Northern Australia. It can thrive even in desert climates and poor soil [7]. In Iraq, *T. terrestris* is used in folk medicine as tonic, aphrodisiac, analgesic, astringent, stomachic, anti-hypertensive, diuretic, lithontriptic and urinary antiinfectives [8]. Hence the purpose of this study was to screen the major phytochemical constituents and evaluate antimicrobial activity of the leaf extracts to support the traditional therapeutic claim and to provide base line information for the scientific communities to carry on further study.

Materials and Methods

All the chemicals and solvents used in experiment were of analytical grade.

Plant collection and identification

Fresh plant parts were collected randomly from local herbal botanical garden of Bhopal, Madhya Pradesh, India. The taxonomic identities of the plant *Tribulus terrestris* was confirmed by botanist Dr. S.S. Khan (Voucher Specimen No: SPTT/010/2010.), Department of Botany, Safia Science College, Bhopal, Madhya Pradesh, India. Fresh plant materials were washed under running tap water, air dried and then homogenized to fine powder and stored in airtight bottles.

Extraction of plant

Aqueous extraction: 10 g of powdered sample was dissolved in 100 ml of distilled water and boiled for 2 h on slow heat. The residue was removed by filtering through 8 layers of muslin cloth; the filtrate was then centrifuged at 5000 g for 10 min. The supernatant was collected and further boiled till the volume was reduced to one-fourth of the original volume of the solvent used [that was 100 ml] giving the concentration of 400 mg/ml [9].

Ethanol extraction: Ten grams of powdered sample was dissolved in 100 ml of ethanol in a conical flask, plugged with cotton wool and then kept on a rotary shaker at 190-220 rpm for 24 h. The supernatant was collected slowly and evaporated in wide mouthed evaporating bowls at room temperature for 2-3 days till the final volume was reduced to one fourth of the original volume of the solvent used [that was 100 ml] giving the concentration of 400 mg/ml [9], and stored at 4°C in airtight bottles.

Phytochemical analysis

Phytochemical analysis of all the samples was determined as follows:

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Molisch's test for Carbohydrates: Five hundred milligram of powdered sample was taken and dissolved in 5 ml of distilled water and then filtered. Filtrate was added with few drops of Molisch's reagent, followed by addition of 1 ml of conc. H₂SO₄ by the side of the test tube. After two minutes, 5 ml of distilled water was added. Red or dull violet colour formation at the interphase of the two layers was taken as positive test [10].

Test for alkaloids: 100 mg of powdered sample was dissolved in 5 ml of methanol and then filtered. Then 2 ml of filtrate was mixed with 5 ml of 1% aqueous HCl. One millilitre of mixture was taken separately in two test tubes. Few drops of Dragendorff's reagent were added in one tube and occurrence of orange-red precipitate was taken as positive. To the second tube Mayer's reagent was added and appearance of buff-colour precipitate was taken as positive test for the presence of alkaloids [11].

Liebermann-Burchard test for steroids: 200 mg of powder sample was dissolved in 2 ml of acetic acid separately; solutions were cooled followed by the addition of few drops of concentrated H₂SO₄. Colour development from violet to blue or bluish-green was taken as positive test steroidal ring [11].

Test for saponins: One gram of powdered sample was boiled in 10 ml of distilled water and then filtered. 3 ml of distilled water was added to filtrate and shaken vigorously for about 5 min. Formation of foam after shaking was taken as a confirmation for the presence of saponins [11].

Shinoda's test for flavonoids: Five hundred milligram of sample was dissolved in 5 ml of ethanol, slightly warmed and then filtered. Few pieces of magnesium chips were added to the filtrate followed by addition of few drops of concentrated HCl. A pink, orange, or red to purple coloration was taken as a confirmation for the presence of flavonoids [12].

Test for tannins: 500 mg of powdered sample was mixed with 10 ml of distilled water and then filtered followed by the addition of few drops of 1% ferric chloride solution. Occurrence of a blue-black, green or blue-green precipitate indicates the presence of tannins [12].

Antimicrobial assay

Bacterial strains: The aqueous and ethanol extracts of *Tribulus terrestris* fruit of 1000 mg/mL, 750 mg/mL, 500 mg/mL and 250 mg/mL concentrations were tested against gram positive *Bacillus subtilis* (ATCC 11778), *Staphylococcus aureus* (ATCC 25923), *Streptococcus epidermidis* (ATCC 24676) and Gram-negative *E. coli* (ATCC 25922), *Shigella flexneri* (ATCC 11435), *Pseudomonas aeruginosa* (ATCC 17440) for their antimicrobial activity. All the bacterial strains were obtained from National Chemical Laboratory, Pune, India. The bacteria were grown in the nutrient broth at 37°C and maintained on nutrient agar slants at 4°C.

Preparation of inoculums (Muller Hinton media): One single colony of each type of microorganism (from the nutrient agar stock culture) was taken with a sterile loop, and was transferred into 10 mL sterile nutrient broth. The broth cultures were incubated in a shaking incubator at 37°C for 16-20 h.

Antibacterial susceptibility test: Disc diffusion assay: The antimicrobial activity of crude extracts of plants was initially assessed against the six tested microorganisms using the agar diffusion method as recommended by the Clinical Laboratory Institute. Nutrient agar medium was prepared by suspending nutrient agar (Merck) 20 g/L

in distilled water. The pH value of the media was adjusted to 7.0, autoclaved and allowed to cool up to 45°C. The media was seeded with 10⁶ CFU/mL prepared inoculums. Subsequently, the seeded medium (75-80 mL) was poured into pre-labelled Petri plates (diameter=14 cm) and allowed to solidify [13].

Each herbal extract reconstituted in DMSO to a concentration of 25, 50, 75, 100 mg/ml (w/v; mg/mL) was dispensed into the discs. The plates were then incubated at 37°C for 24 h for bacteria after which microbial growth was determined by measuring the diameter of the inhibition zone (mm) using a ruler or caliperscale. Each experiment was performed in triplicate and repeated twice. Standard antibiotics (5 µg) Gram-positive (TE-tetracycline, OF-ofloxacin, AZ-azithromycin, PC-piperacillin) and Gram-negative FU nitrofurantoin, GM-gentamicin, CX-cefotaxime, NF norfloxacin, (5 µg/disc) were prepared as positive control. Pure dimethyl sulfoxide (99.9%) was used as negative control.

Results and Discussion

Phytochemical screening

As it is clearly indicated in the Table 1 below, preliminary phytochemical analysis showed alkaloids, saponins, carotinoid and tannins detected in petroleum extract while phytosterols and saponins detected in aqueous and ethanol extract.

Antibacterial activity

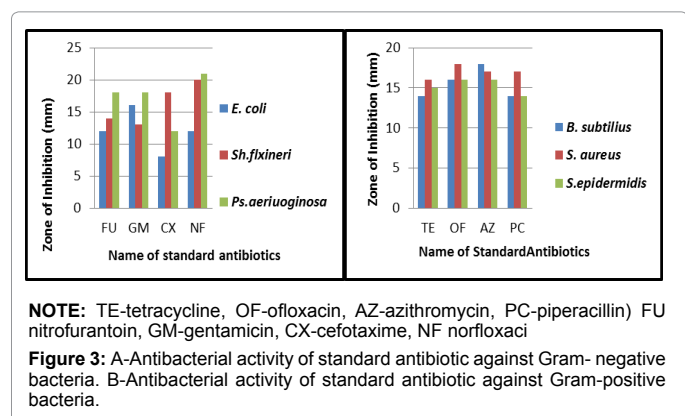
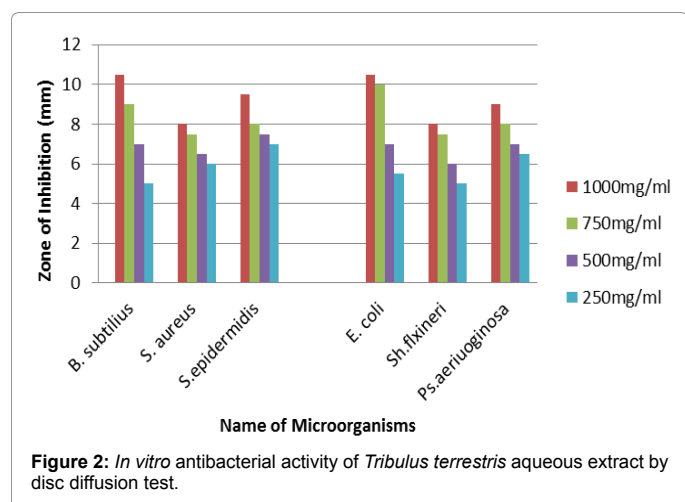
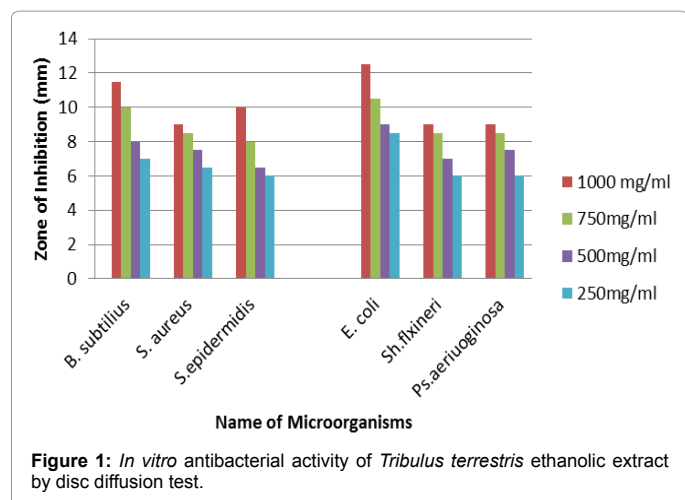
Antimicrobial activity of *Tribulus terrestris* fruit extracts in two different solvents has been tested against Gram positive and Gram negative bacterial strains. All figures summarize the microbial growth inhibition of aqueous and ethanolic extracts of *Tribulus terrestris*.

The results indicated that the different extracts of *Tribulus terrestris* exhibit antibacterial activity. *Tribulus terrestris* ethanolic extracts has shown higher activity than aqueous extract (Figures 1 and 2). Bacterial strains were tested in 25, 50, 75, and 100 mg/ml ethanolic and aqueous extracts for 24 hrs by disc diffusion assays. The poor activity at lower concentrations (25-50 mg/ml) may be attributed to the less solubility

S.No.	Name of Tests	Tests/Reagents	TTAE	TTEE
1	Reducing Sugar	Fehlings test	+	+
		Molish test	+	+
2	Glycosides	Borntrager's	+	+
3	Alkaloids	Dragendorff's test	-	+
		Mayer's test	-	+
4	Phytosterol	chloroform	-	+
5	Steroidal compounds	Salkowski's Test	-	+
		Lieberman's Test	-	+
6	Saponins	Froth test	+	+
7	Tennins	Ferric chloride test	+	+
		Formaldehyde test	+	+
		Test for phlobatanins	+	+
8	Fixed oils and Fats	Spot test	-	-
9	Flavonoids	Test for free flavanois	+	+
		Lead Acetate Test	+	+
		Sodium Hydroxide	+	+
10	Phenolic compound	Ferric chloride test	+	+
11	Protein and Amino acid	Biuret Test	-	+
		Ninhydrin Test	-	+

Here, +: presence, -: absence, TTAE=*Tribulus terrestris* aqueous extract, TTEE=*Tribulus terrestris* ethanol extract

Table 1: Phytochemical analysis ethanolic and aqueous fruits extract of *Tribulus terrestris*.



of its active compounds, which may improve with stringent extraction procedures. At higher concentration (75-100 mg/ml) all the extracts showed significant antimicrobial activity.

It was observed that all extracts showed growth inhibition of various clinical isolates. Zone inhibition of all tested samples for Gram positive and Gram negative bacterial strains were compared to standard antibiotics which is broad spectrum antibiotic (Figure 3).

Result of the present study indicated that the alkaloid extracts of

all the parts of *T. terrestris* have activity against both gram-positive and gram-negative bacteria indicative of the presence of broad spectrum antibiotic compounds. Many workers have reported on the antibacterial activity of natural products obtained from various sources of plant materials such as seeds [14], roots [15], plant parts [16-19] etc. Great attention is directed towards isolation, identification and synthesis of the natural products active against a wide variety of bacteria and fungi that cause diseases both in humans and animals. In this direction, the antimicrobial activity of ethanolic aqueous extract of *Tribulus terrestris* herb was tested against four species of bacteria namely gram positive organisms like *Bacillus subtilis*, *Staphylococcus aureus*, *S. epidermidis* and gram negative organisms like *E. coli*, *P. aeruginosa*, and *Sh. Flexneri*.

In the present investigation, the antimicrobial activity of various test samples in comparison with standard antibiotics were determined and found to proceed in a dose-dependent manner for different bacterial strains. Those are becoming a clinical problem in hospital patients. Gram-positive bacteria, *S. aureus* is known to cause serious diseases such as pneumonia, meningitis etc., in hospital patients [20-23]. *E. coli* and *P. aeruginosa* cause the Urinary Tract Infections (UTI), pulmonary tract infections, burns, wounds, dysentery-like diarrhoea and other blood infections and similar also true for *S. epidermidis* [24].

Conclusion

This study clearly indicates that extracts of the plants studied possess potent antimicrobial activity. The use of crude drug of such plant as an agent to control microbial pathogens needs further extensive research for their better economic and therapeutic utilization. Furthermore, it may help to discover new chemical classes of antibiotics that could serve as selective agents for the maintenance of animal or human health and provide biochemical tool for the study of infectious diseases. The presence of most general phytochemicals might be responsible for their therapeutic effects. It further reflects a hope for the development of many more novel chemotherapeutic agents or templates from such plants which in future may serve for the production of synthetically improved therapeutic agents.

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